

A DNA marker for *ppd*, a gene conferring insensitivity to photoperiod in common bean

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Genes responding to photoperiod have a major influence on partitioning of photosynthate (Wallace et al., 1993a, b) which, in turn, controls the level of all three major physiological components of yield: biomass, harvest index, and days to maturity. Therefore, identification and use of genetic "tags" for photoperiod genes may be particularly helpful for increasing the efficiency of breeding for traits such as higher biomass, higher harvest index, and early maturity.

A single locus, *Ppd*, is responsible for the difference in photoperiod sensitivity between two common bean varieties, Redkote (*Ppd/Ppd*: photoperiod sensitive) and Redcloud (*ppd/ppd*: photoperiod insensitive) (Wallace et al., 1993a). Both varieties are determinant (*fin/fin*). The CIAT line, Mam4, also is photoperiod sensitive but is indeterminant in growth habit. We used recombinant inbred lines derived by single seed descent from Redcloud x Redkote and Redcloud x Mam4 to identify a DNA marker linked to the photoperiod gene and locate *Ppd* on the bean linkage map. In addition, other progenies and varieties were tested to determine if the marker was useful for identifying *ppd* in a wider germplasm.

Materials and methods: F8 recombinant inbred lines (RILs) from Redcloud x Redkote and from Redcloud x Rojo 70 were developed at Cornell University. F7 progenies from Redcloud x Mam4 were developed at CIAT. Seed of other varieties tested (San Martin, Rojo 70, Rabia de Gato, and VRB81047) were available at Cornell University. Seeds were germinated in artificial soil and sand in a 1:1 ratio. One week after germination young leaf tips were removed for DNA extraction using the method of Torres et al. (1993).

Over 300 short oligonucleotide primers of arbitrary sequence were screened using the RAPD technique of Williams et al. (1990). The primary screen consisted of six samples: Redcloud, Redkote, two sensitive RILs from the Redcloud x Redkote cross and two insensitive lines from the same cross. Each reaction mixture contained 0.2 uM oligonucleotide primer, 0.13 mM of each of the four dNTPs, 10 ng of template DNA, 2.5 ul 10X buffer, 1.5 mM MgCl₂, 0.5 unit of *Taq* polymerase (Promega), and sufficient sterilized distilled water to make the total volume 25 uL. The cycle profile was 94° for 1 min, 35° for 2 min, and 72° for 2 min, respectively, with a 6 min extension at 72° after forty cycles. Amplified products were separated on a 1% NuSieve (FMC Bioproducts):1% agarose gel, visualized with 0.01 mg/mL ethidium bromide and photographed. Primers that displayed consistent RAPDs between the sensitive and insensitive genotypes were further tested on the remaining lines.

Results and discussion: The 10mer primer, P5 (sequence 5'-TCTCTGTCCC-3') amplified a 1.6 kb fragment (designated P5b) in Redcloud and most of the photoperiod insensitive RILs. Analysis of all 58 RILs gave only three recombinants between P5b and *Ppd*. A similar examination of the 48 RILs from Redcloud x Mam4 gave only two recombinants between the marker and *Ppd*. In addition, P5b displayed close linkage with *Fin* (two recombinants in 48 lines). These data indicate that P5b is located about midway between *Ppd* and *Fin*, and the recombination frequency agrees well with the 15 cM estimated distance between *Ppd* and *Fin* (Gniffke, 1985).

When primer P5 was used to amplify DNA from other bean lines, all four (San Martin, Rojo 70, Rabia de Gato, and VRB81047) produced the P5b fragment. As the former two lines are

photoperiod sensitive while the latter two are insensitive, the P5b fragment does not appear to be particularly diagnostic for the *ppd* gene. Similarly, there appeared to be little correlation between P5b and the *Fin* locus when comparing unrelated lines.

References

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